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RESEARCH ARTICLE

Acute toxicity, anti-inflammatory and antinociceptive investigations of extracts from *Ampelocissus africana* (Lour) Merr (Vitaceae) rhizomes

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Abstract

Ampelocissus africana (Lour) Merr (Vitaceae) is a plant used in traditional medicine in Burkina Faso in the treatment of wounds, edema, drops, infections. This study focused on pharmacological investigations effects of methanolic and aqueous extracts from *Ampelocissus africana* (Lour) Merr rhizomes.

The acute toxicity of the extracts from *Ampelocissus africana* was performed under OECD 423 guidelines. The anti-inflammatory activity was evaluated in vivo using carrageenan-induced paw edema of mice at the doses to 50, 100, 200, 400, 600 mg/kg body weight (bw). The antinociceptive activity of the extracts was determined using acetic acid, formalin nociception models in mice at the doses to 50, 100, 200, 400 and 600 mg/kg bw. The involvement of ATP-sensitive K^+ channel pathway and opioid system were tested using glibenclamide, and naloxone respectively at the unique dose (400 mg/kg).

No mortality of mice were observed at dose of 2000 mg/kg b.w. The lethal dose (LD_{50}) value estimated to 5000 mg/kg b.w. The tests were showed that the extracts exerted significant dose-dependent anti-inflammatory responses in the paw induced by carrageenan (from 37 to 72.90 percent inhibition), antinociceptives in acetic-induced abdominal contractions (from 25.10 to 63.08%), and in formaldehyde-induced paw licking (from 9.27 to 71.77%) tests. The pretreatment with naloxone and glibenclamide did not affect the antinociceptives effects.

The results suggested that the extracts from the rhizomes of *Ampelocissus africana* possessed anti-inflammatory and antinociception effects in mice. These effects could justify the uses of this plant in traditional medicine.

Keywords: Ampelocissus africana; acute toxicity; inflammation; analgesic

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Introduction

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against external or internal stimuli. It is a local or systemic tissue reaction that aims to eliminate the harmful agent, inhibit its further spread and, possibly, repair damaged tissue. Although this is a defense mecha-

nism, uncontrolled and persistent inflammation can act on complex events and involve to the synthesis of proinflammatory mediators such as prostaglandins, leukotrienes and cytokines (TNF α, IL1β, IL6) [1]. This inflammatory process and pain can then cause a lot of suffering and discomfort to the victims, lowering the quality of life and therefore need to be managed [2].

The synthetic chemicals such as non-steroidal anti-inflammatory drugs (NSAIDs) were widely prescribed all over the world against inflammatory disorders and pain [3]. However, currently used anti-inflammatory drugs in prolonged use are associated with some asymptomatic relief and the greatest drawback lies in their toxicity to liver, kidney and gastrointestinal linings [4]. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary [5]. The use herbal medicine is gaining support and recognition across the world because most of these products are believed to have bioactive compounds responsible for healing various diseases without any side effects and at a lower cost. Although, *Ampelocissus africana* (Lour) Merr (Vitaceae) is traditionally used in the treatment of many types of pain such as drops, rheumatism, edemas, old wounds, circulatory disorders, analgesic and inflammatory conditions [6].

In Burkina Faso, no scientific report is available to validate these traditional uses. It is in this context that present study was designed to evaluate the anti-inflammatory and analgesic activities as well as acute toxicity of rhizomes extracts of *Ampelocissus africana*.

Material and methods

Plant collection and sample préparation

The rhizomes of *Ampelocissus africana* (Lour) Merr were collected in September 2019 around Dedougou, Burkina Faso. The plant was authenticated at Burkina Faso National Herbarium (HNBU) within the National center for Scientific and Technological Research (CNRST) where the voucher specimen (N° 8754) was deposited.

The rhizomes of the plant were cut into small pieces before being dried at room temperature away from the sun. The material was then reduced to the fine powder and stored in a hermetically freezer bag until use in order to preserve all their physicochemical properties.

Chemicals reagents

Ketamine, acetaminophen, acetylsalicylic acid (ASA), glibenclamide, naloxone, carrageenan, were purchased from Sigma® (St Louis, USA). Acetic acid from Prolabo and formol from Cooper. Tramadol was purchased at a local pharmacy. All solvents used were of analytical grade.

Animals and ethical approval

The NMRI mice weighing 25 $\pm$ 5 g (both sexes) were obtained from the animal's house of Institute of Health Sciences Research. All the animals were maintained in appropriate cages at 23 $\pm$ 2°C, with 12 hours light / 12 hours dark cycle. The tests were performed strictly according to the guidelines of laboratory animals care and the ethics guidelines for painful experimentation on conscious animals [7]. Animals were fasted for 16 hrs with access to water and weighed before the experiments (anti-inflammatory and antinociceptives tests). The laboratory experimentation was carried out according to the experimental protocols validated by the Institute of Health Sciences Research laboratories and meeting the international standards in this field (guidelines established by the European Union on the protection of animals, CCE Conseil 86/609).

Extraction

• **Methanolic extraction**: 100 grams of powder was soaked in 1 liter of methanol during 24 hours. Then, whatman's filter paper No.1 was used to filter the mixture. The filtrate was concentrated to dryness in vacuum using the rotary evaporator at a temperature of 40°C and the concentrate was kept in stove until the methanol has completely evaporated.

• **Aqueous extraction**: 100 grams of powder was soaked in 1 liter of distilled water during 24 hours. After filtration the mixture was centrifuged at 10 000 trs/min for five minutes and frozen to be lyophilized. All of these extracts (methanolic and aqueous) were preserved and used for different tests.

Acute toxicity test

The acute toxicity study was conducted according to the acute toxic class method of the Organization for Economic Cooperation and Development (OECD, 2001) test guideline 423 [8]. The female's mice were randomly into three batches with three mice / group. The test was carried out twice After four hours fasting with access to running water, distilled water at a dose of 10 mL / kg and the extracts (aqueous and methanolic) at a single dose of 2000 mg/kg of body weight were administered orally by gavage to the animals. The animals were observed continuously during two hours after the treatment and next, they were fed. They were observed daily during 14 days and all signs of toxicity and mortality have been noted. The animals were weighed on the day of the start of the study, 24 hours, 48 hours, 72 hours after, 7^{th} day and on the 14^{th}day at the same time. At the end of this test,

the mice were sacrificed and organs such as liver, spleen, kidney, lung, and heart were collected, observed and weighed.

In vivo anti-inflammatory activity

carrageenan-induced paw edema test The method described by [9] with minor modifications by [10] was used to carry out the anti-edematous activity of extracts of *Ampelocissus africana*. The edema was induced by injection of 0.05 mL of 1% carrageenan (NaCl 0.9%) into the right hind paw of mice. One hour before the injection of carrageenan, groups of six NMRI mice were each treated orally either with the extracts (methanolic or aqueous) at doses of 50, 100, 200, 400, 600 mg/kg bw, either acetylsalicylic at a dose of 100 mg/kg bw, or distilled water at 10 mL/kg bw. The volume of the right paw was measured 1 hour before then 1, 3, 5 hours after the injection of carrageenan using a plethysmometer (Ugo Basile No. 37140).

% Inhibition = [(Ac - At) / Ac] X 100

Ac, At : average difference of the volume increase of the paw of mice to the control group and treated groups respectively

Analgesic activities

Acetic acid-induced writhing test The analgesic effect of the extracts was determined by the number of abdominal contortions induced by the intraperitoneal injection of acetic acid (0.6 %) according to the method described by [11]. Groups of six mice each were formed. The mice received either extracts (aqueous or methanolic) at doses of 50, 100, 200, 400, 600 mg / kg bw, or distilled water at a dose of 10 mL/kg bw for the white control batch and acetaminophen at a dose of 200 mg / kg for the reference batch. 1 hour after oral administration of the substances, acetic acid was injected into the peritoneum of the mice at a dose of 10 mL/kg and after five minutes, the number of writhes was counted in each mouse for 15 minutes. The percentage inhibition of the number of contortions in mice performed was calculated against the blank by the formula below:

% Inhibition = [(Wb-Wt) / Wb] $\times$ 100

Wb, Wt: Average of the number of writhings of the mice in the white control batch and the treated batch respectively

Analysis of possible mechanism of action of *Ampelocissus africana:* Involvement of ATP-sensitive K^+ channels pathway

For the study of the possible contribution of the ATP-sensitive K^+ channels of the extracts, the method described by [12] was used. To do this, the batches of six mice were pretreated orally with glibenclamide 10 mg/kg bw (an ATP-sensitive K^+ channel inhibitor). 15 min after treatment with glibenclamide, the extracts (aqueous and methanolic) at a dose of 400 mg/kg bw were administered to the treated group, and the vehicle at 10 mL/kg bw to the white control group. Sixty minutes later, the animals were assessed using abdominal contortions induced by acetic acid (0.6%) and after five minutes, the number of contortions was recorded in each mouse for 15 minutes. The percentage inhibition of the number of contortions in mice performed was calculated against the blank by the formula:

% Inhibition = [(Wb-Wt) / Wb] $\times$ 100

Wb, Wt: Average of the number of writhings of the mice in the white control batch and the treated batch respectively

Formalin-induced paw licking test

The antinociceptive activity of the extracts was achieved using the formalin test described by [13] with slight modifications. The test batches were treated with the extracts (aqueous and methanolic) at doses of 100, 200, 400 and 600 mg / kg bw; the reference batch, was treated with tramadol at 10 mg/kg bw and the white batch, distilled water at 10 ml/kg orally. The lots consisted of six NMRI mice each. 1 hour after administration, 20 μL of formalin 2.5% (0.9% NaCl) was injected into the sub plantar area of the right hind paw. Immediately after the injection of the formaldehyde solution, the time spent licking or biting the injected paw was counted for 5 minutes (early phase), then between 15 and 30 minutes (late phase). The analgesic effect was determined based on the following formula:

% Inhibition = [(Tb-Tt) / Tb] $\times$ 100

Tb, Tt : licking time (in seconds) of the mice in the white group and the treated group respectively

Analysis of possible mechanism of action of *Ampelocissus africana* : Involvement of opioidergic pathway

The method described by [14] was used to investigate the possible implication of the opioidergic pathway of *Ampelocissus africana*. Groups of six mice each were made up for this analysis. The test groups were treated with the extracts (aqueous, methanolic) at 400 mg/kg bw, the white control group received distilled water at 10 mL/kg bw. Mice were pretreated with naloxone 2 mg/kg, (a non-selective opioid receptor antagonist) 15 minutes before administration of the test solutions. 1 hour later, all mice were subjected to the test of formalin test. Immediately after the injection of formaldehyde solution, the licking time was counted for 5 minutes, then between 15-30 minutes and the percentage inhibition was determined by the formula:

% Inhibition = [(Tb-Tt) / Tb] $\times$ 100

Tb, Tt : licking time (in seconds) of the mice in the white group and the treated group respectively

Statistical analysis

The data were expressed as Mean ± Standard Error of Mean (SEM). The statistical analysis was carried out using one-way ANOVA followed by the Bonferroni multiple comparison test on Graph Pad Prism software version 6.0. The level of significance was accepted at $p < 0.05$ compared to the control and between treated groups.

Results and discussion

Acute toxicity test

The administration of the extracts from *Ampelocissus africana* at a single dose of 2000 mg/kg did not show any abnormal behavior during the 2 hours post-treatment. No mortality was noticed 3 days later. The extracts of this plant is classified to the 5^{th} toxicity class with low oral acute toxicity and a LD_{50} value estimated to 5000 mg/kg b.w [8, 15].

The weight of the animals recorded on the day of the start of the test (D0) and one day (D1), 2 days (D2), 3 days (D3), one week (D7), two weeks (D14) after was presented in the table 1. The body weight of the animals increased slightly in the treated batches as in the control. These results suggest that the extracts did not have a toxic effect on the treated mice. The weight of the organs of the treated animals is not significantly different compared to the control. Macroscopic analysis of the organs (heart, spleen, kidneys, lungs, liver) of the mice after 14 days did not showed any signs of alteration. On the other hand, the administration of the extracts did not influence the relative weight of the vital organs removed at the end of the test (Table 2). The study of the relative organs weight showed that the extracts had no toxic effects.

Carrageenan-induced paw edema test

The anti-inflammatory activity of the extracts was estimated at doses of 50, 100, 200, 400, 600 mg/kg bw against the paw edema induced by carrageenan. Both extracts significantly reduced edema in a dose-dependent manner with a high percentage of inhibition of edema observed at the fifth hour (Fig.1a, Fig.1b) ranging from 69.75 for aqueous extract to 72.90% for methanolic extract. At the same dose (100 mg / kg), the effect of the extracts (54.55% for the aqueous extract and 56.95% for the methanolic extract) was less active than acetylsalicylic acid (86.18%) used as a reference. The efficient dose (ED_{50}) values of the methanolic and aqueous extract were respectively 61 mg/kg and 97 mg/kg bw.

The inflammatory response induced by carrageenan is considered a biphasic model. The early phase of edema that develops in the paws of mice after injection of carrageenan lasts 90 minutes and is mediated by the release of histamine and serotonin and kinins. The second phase corresponding to the last 5 hours is due to the release of prostaglandins [16, 17]. According to [18], nitric oxide (NO) is a mediator contributing to the production of an inflammatory response in inflammation induced by carrageenan.

Acetic acid-induced writhing test

The effect of oral administration of extracts using the abdominal contortion test in mice was shown in Fig. 2. It was found that both of extracts from A. africana at doses of 50, 100, 200, 400 and 600 mg/kg was able to reduce significant dose-related the visceral nociceptive effects induced by acetic acid compared to the control group. The percentage of inhibition was greater than 50% at the dose of 200 mg /kg for the methanolic extract and 200 mg/kg for the aqueous extract. The reference used (paracetamol) had a percentage inhibition at the dose of 200 mg / kg higher than the extracts (70.85%). The ED_{50} values of the methanolic and aqueous extract of 183 mg/kg and 358 mg/kg bw respectively.

The acetic-induced writhing test is a method used to estimate peripheral antinociceptive activity [19]. The injection of acetic in mice would lead to an increase in the level of cyclooxygenase (COX) and products of lipoxygenase (LOX) thus stimulating the peripheral nociceptive neurons in the peritoneal cavity by causing pain sensations by the release of various mediators inflammatory drugs such as bradykinin, serotonin, histamine, substance P or prostaglandins (PGE2 and PGF2) [20]. The action of the extracts could be partly linked to the inhibition of COX and/or LOX and other inflammatory mediators in peripheral tissues. The nociceptive response elicited by acetic is also dependent on the release of certain cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 and interleukin-8 by modulation of macrophages and mast cells located in the peritoneal cavity [14].

Involvement of ATP-sensitive K+ channels pathway

To assess a possible antinociceptive mechanism including involvement of ATP-sensitive of A. africana extracts, we examined the effects of pretreatment of glibenclamide (an ATP-sensitive potassium channel antagonist) in the acetic acid-induced abdominal constriction test. The results in Fig.3 showed that the administration of glibenclamide (10 mg/kg) alone did not significantly affect abdominal contortions assessed by injection of 0.6% acetic acid. The pretreatment with glibenclamide was not able to reverse the antinociception effect promoted by the extracts from A. africana, with a dose of 400 mg/kg. This study

Table 1 Mortality and body weight (g) of mice

Extracts	Control 1^{st} test	AAM	AA A	Control 2^{nd} test	AA M	AA A
Mortality (72 H)	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**	**0/3**
Day 0	32.15 ± 0.3	27.28 ± 0.9	28.81 ± 0.4	33.68 ± 2.3	24.88 ± 0.4	31.21 ± 0.9
Day 1	32.48 ± 0.5	26.63 ± 1.3	28.86 ± 0.4	34.74 ± 1.7	25.63 ± 0.9	31.18 ± 0.7
Day 2	32.67 ± 0.4	27.31 ± 1.6	28.33 ± 0.4	35.33 ± 2.4	25.98 ± 1.1	30.67 ± 0.9
Day 3	32.42 ± 0.8	27.66 ± 1.5	29.5 ± 0.5	35.17 ± 2.6	25.99 ± 1.2	30.82 ± 0.3
Day 7	31.54 ± 1.2	29.69 ± 1.6	28.89 ± 0.3	33.11 ± 1.8	26.93 ± 1.6	29.68 ± 1
Day 14	34.74 ± 0.6	33.18 ± 1.08	30.94 ± 0.5	34.59 ± 1.5	28.97 ± 2	32.33 ± 0.3

Mean and standard deviation were presented (n = 3)
AA M: *Ampelocissus africana* methanolic extract; AA A: *Ampelocissus africana* aqueous extract

Table 2 Mean relative organs weight (%) of mice

Extracts	Control 1^{st} test	AAM	AA A	Control 2^{nd} test	AA M	AA A
Heart	0.53 ± 0.08	0.52 ± 0.04	0.49 ± 0.01	0.49 ± 0.08	0.47 ± 0.03	0.48 ± 0.03
Spleen	0.5 ± 0.13	0.54 ± 0.18	0.51 ± 0.11	0.41 ± 0.09	0.4 ± 0.04	0.41 ± 0.07
Kidneys	1.15 ± 0.06	1.12 ± 0.08	1.14 ± 0.09	1.06 ± 0.05	1.08 ± 0.06	1.10 ± 0.06
Lung	0.79 ± 0.03	0.82 ± 0.15	0.81 ± 0.12	0.7 ± 0.06	0.68 ± 0.09	0.71 ± 0.09
Liver	5.15 ± 0.19	5.46 ± 0.5	4.85 ± 0.04	4.54 ± 0.14	5.35 ± 0.29	4.68 ± 0.22

Mean and standard deviation were presented (n = 3)
AA M: *Ampelocissus africana* methanolic extract; AA A: *Ampelocissus africana* aqueous extract

appeared at the extracts effect (400 mg / kg) and glibenclamide (10 mg / kg) treatments.

Reports [21] that glibenclamide specifically blocked the ATP-sensitive K^+channel, without effects on other types of K^+ chan-nel such as Ca^{2+}activated K^+ channel and voltage-gated potas-sium channels. The opening of the K^+ channel sensitive to ATP would be followed by an efflux of K^+ions and removal excitabil-ity of the cell membrane by hyperpolarization and/or repolariza-tion [22].

Formalin-induced paw licking test

The extracts at doses of 100, 200, 400, 600 mg/kg produced a dose-dependent inhibition of the licking response induced by formalin in both phases: neurogenic phase (0 to 5 min) and inflammatory phase (15 at 30 min) compared to the control group as shown in Fig. 4. However, the antinociceptive effect was more pronounced in the second phase. Tramadol (10 mg/kg) effectively reduced formalin-induced nociception in both phases (1^{st}phase: 70%, 2^{nd}phase: 88.67%). ED_{50} values of 354 mg/kg and superior 600 mg/kg bw respectively for the 2^{nd} phase. The methanolic extract caused a strong inhibition at a dose of 600 mg / kg (66.44% for the 1^{st} phase and 71.77% for the 2^{nd} phase.

The formalin model of nociception is described as a method that discriminates pain in its central and / or peripheral components [23]. This model makes it possible to study two types of pain with an early phase (0-5 min after injection of formalin) corresponding to intense neurogenic pain with the involvement of mediators such as substance P, bradykinin, histamine and serotonin (evidence of direct stimulation of nociceptors). The late phase (15-30 min) involves the production and release of prostaglandins, bradykinin, histamine, serotonin, tachykinins and glutamate by cells activated by formalin [24].

Involvement of opioidergic pathway

To investigate the possible antinociceptive mechanism on formalin test, animals were pretreated with naloxone (2 mg/kg). Naloxone is a non-selective antagonist at δ, μ, and K opioid receptors (with an affinity for the μ receptor) which plays a major

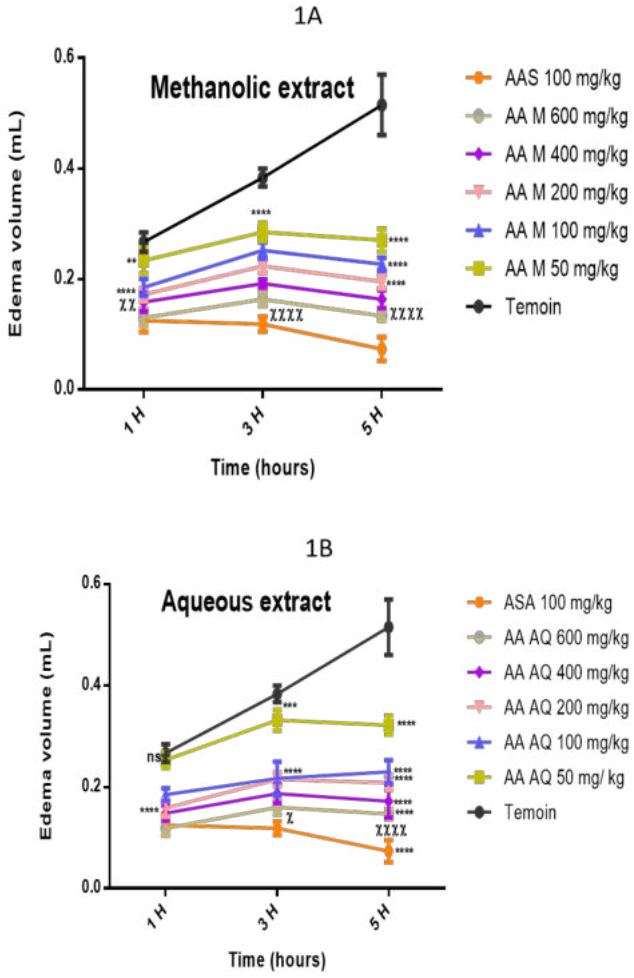

Figure 1 Effects of Ampelocissus africana extracts (Fig.1.A and Fig.1.B) on carrageenan-induced edema Values are mean ± S.E.M. n = 6. Data was analysed using Two-way ANOVA followed by Bonferroni multiple comparison test, ns = no significance P > 0.05, ** = P < 0.01, **** = P < 0.0001 indicate significance compared from control group; (áţą) = P < 0.05, (xx) = P < 0.01, (xxxx) = P < 0.0001 vs reference group (acetylsalicylic acid)

role in central nociceptive system [25, 26]. For these extracts at a dose of 400 mg/kg were used. The results showed that the administration of naloxone alone did not affect paw licking time. Together taken, naloxone 15 min before and extracts did not reverse the antinociception. Naloxone were not able to reverse the antinociceptive effect of extracts in mice. This suggests that the activation of opioid receptors may not be involved in these effects.

Inflammation is a complex process. It is therefore necessary to use various models of inflammation, involving the analysis of several factors, to assess an anti-inflammatory activity of a substance [27].

These potentialities of *A. africana* rhizomes could be exploited in the prevention of diseases with an inflammatory component.

Conclusion

All of the results to this study suggest that the rhizomes of *Ampelocissus africana* (Lour) Merr rhizomes possesses anti-inflammatory and antinociceptive properties and can be used safely orally. That could justify its traditional uses in the treatment of many conditions including diseases with an inflammatory component. These results obtained the scientific basis for the use of *Ampelocissus africana* to prevent and treat inflammatory processes.

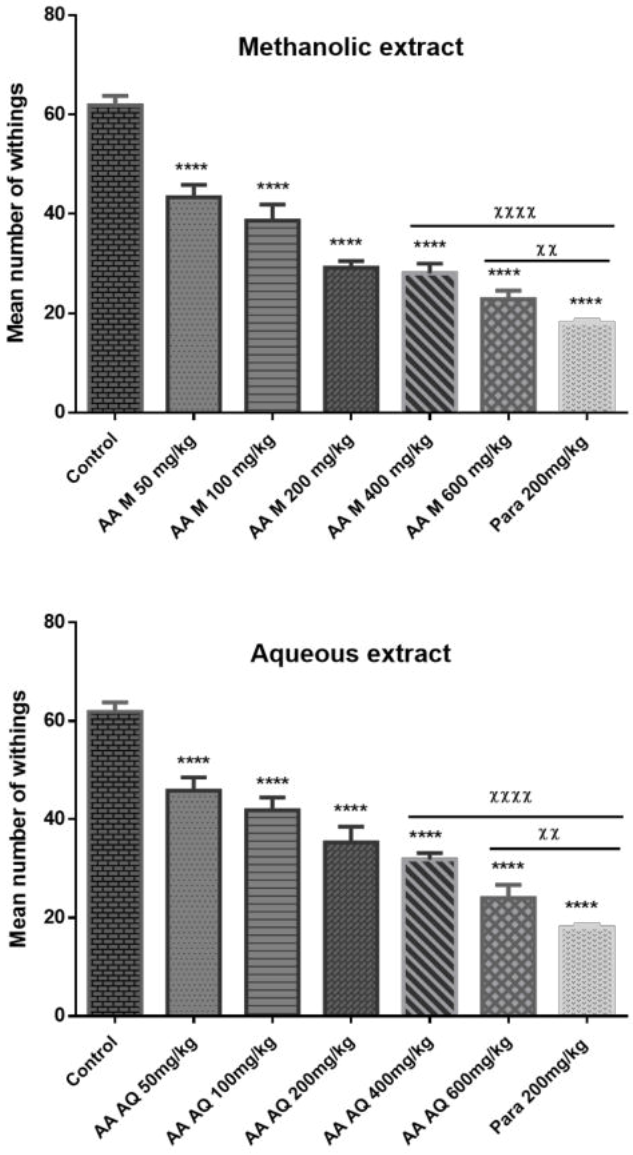

Figure 2 Effects of *Ampelocissus africana* extracts on acetic acid-induced abdominal Writhes in mice Each column represents the Mean ± SEM, n = 6. Data was analysed using One-way ANOVA followed by Bonferroni multiple comparison test,**** = P < 0.0001 indicate significancecompared from control group; (xx) = P < 0.01, (xxxx) = P< 0.0001 vs reference group (paracetamol)

Authors' contributions

WLME. B-K carried out the work, performed the statistical analysis, interpreted the results and drafted the manuscript. **N. O** conceived the study, and participated in its design and coordination and helped to draft the manuscript. **A. C-C** contributed to perform antiinflammatory and antinociceptives tests. **M. N-T** participated in the acute toxicity and formalin tests. **T K. T** participated in the antiinflammatory test and contributed to adapt the protocol of antinociception. **S. I** supervised the acute toxicity test. **CB. A** and **AGL. B** contributed to perform antinflammatory and the implication of K + channels and opioid receptors tests. **M. L**, **M. K**, **S. O** contributed to analyze the results, read and approved the manuscript. All authors read and approved the final manuscript

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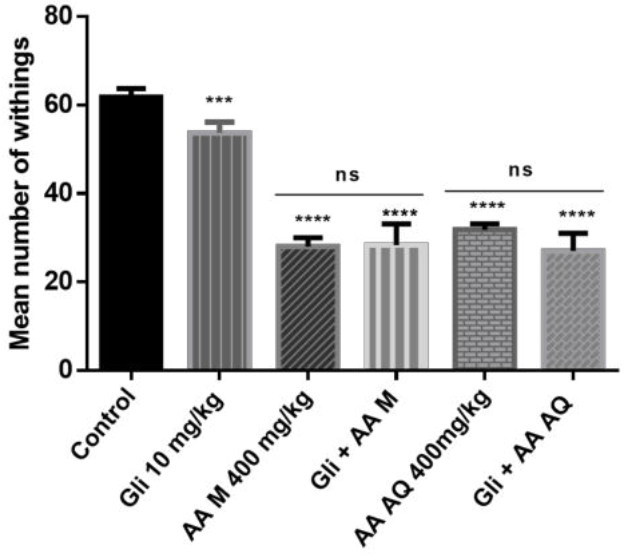

Figure 3 Effects of glibenclamide (Gli) on analgesic activity of *Ampelocissus africana* extracts AA M: *Ampelocissus africana* methanolic extract; AA A: *Ampelocissus africana* aqueous extract
Data are Mean ± SEM, n = 6, analysed using One-wayANOVA followed by Bonferroni multiple comparison test, *** = P < 0.001, ****= P < 0.0001 indicate significance compared from control group, ns = no significance P > 0.05 extract vs pretreatement with Glibenclamide.

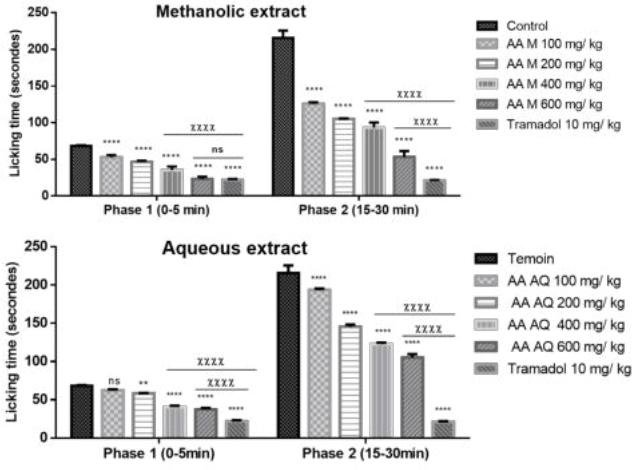

Figure 4 Effects of Ampelocissus africana extracts (Fig.4.a and Fig.4.b) in the formalin-induced paw licking test Each column represents the mean ± S.E.M, n= 6, ***P < 0.001 comparedwith control group, (One-way ANOVA followed by Bonferroni multiple comparisontest), **** = P < 0.0001 indicatesignificance compared from control group, ns = no significance P > 0.05, (xxxx) = P< 0.0001 vs reference group (tramadol)

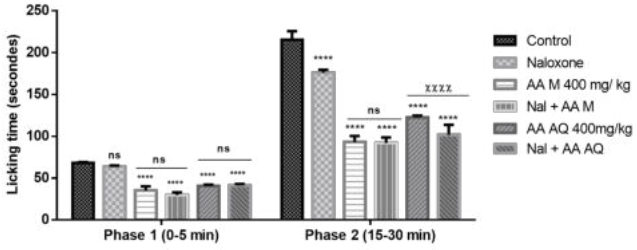

Figure 5 Effects of Naloxone (Nal) on analgesic activity of *Ampelocissus africana* extracts AA M: *Ampelocissus africana* methanolic extract; AA A: *Ampelocissus africana* aqueous extract
Data are Mean ± SEM, n = 6, (One-way ANOVA followed by Bonferroni multiple comparison test), **** = P < 0.0001 indicate significance compared from control group, ns = no significance P > 0.05, (xxxx) = P < 0.0001 extract vs pretreatement with Naloxone.

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